

In the Specification:

Please replace the paragraph beginning at page 5, line 22, with the following:

--Fig. 1 shows that HBsAg335-343, WLSLLVPFV (SEQ ID NO:10), is the minimal optimal CTL epitope recognized by CTL stimulated HBsAg329-348. A CTL clone from patient A-1 and a CTL cloned line from patient A-3, generated by stimulation with HBsAg329-348, were tested against JY target cells prepulsed either with truncations (SEQ ID NOS:22 and 28-39, respectively) (upper panels) or with overlapping 9-mers or 10-mers (SEQ ID NOS:22, 40-50, 15, 10, 16, 51-55, 25, 56, 38 and 39, respectively) (lower panels) covering HBsAg329-348.--

Please replace the paragraph beginning at page 5, line 29, with the following:

--Fig. 2 further confirms that an optimal epitope within HBsAg329-348 for in vitro CTL induction is HBsAg335-343 (SEQ ID NOS:22, 53, 52, 10, 51, 50 and 25, respectively).--

Please replace the paragraph beginning at page 6, line 3, with the following:

--Fig. 4 shows the results of HLA-A2.1 competitive binding inhibition assays (SEQ ID NOS:24, 7, 9, 10, 25, 26 and 27, respectively), represented as % inhibition of HBcAg18-27 specific lysis in a 4 hour ⁵¹Cr release assay.--

Please replace the paragraph beginning at page 33, line 23, with the following:

--Two HLA-A2 positive patients with acute hepatitis (A-1 and A-3) were initially selected for analysis of the CTL response to HbsAg329-348 (ASARFSWLSLLVPFVQWFVG (SEQ ID NO:22)), which contains 2 overlapping HLA

a2.1 allele specific binding motifs (WLSLLVPFV (SEQ ID NO:10) and LLVPFVQWFV (SEQ ID NO:25)). One of these patients (A-3) was known from previous experiments to display an HLA A2 restricted CTL response to a 10 residue HBV nucleocapsid epitope (HBcAg18-27) that also represents an HLA A2.1 allele specific binding motif (FLPSDFFPSV (SEQ ID NO:23)). This patient was considered a potential responder to one or both of the motifs in HBsAg329-348. Another patient (A-1), known to be a nonresponder to HBsAg18-27, was studied for comparison.--

Please replace the paragraph beginning at page 35, line 6, with the following:

--The results, shown in Fig. 1, indicated that only the first of the HLA-A2.1 binding motifs (HBsAg335-343) is recognized by the CTL. Furthermore, the data demonstrate that this peptide (WLSLLVPFV (SEQ ID NO:10)) is the minimal, optimal HLA-A2 restricted epitope recognized by HBsAg329-348 stimulated CTL, since omission of the extreme amino-terminal or the extreme carboxy-terminal residue from HBsAg335-343 abolishes recognition by the CTL.--

Please replace the paragraph beginning at page 37, line 22, with the following:

--Nucleotide sequence analysis of circulating virion DNA in acutely infected patients showed that all patients, including the CTL nonresponders, were infected by viruses that expressed the precise amino acid sequence present in the prototype HBsAg335-343 peptide used to stimulate expansion of CTL in vitro. Since residues 335-343 are known to be conserved in all the published HBV sequences derived from all 4 HBV subtypes, as published in the GenBank-72 database, as well as in the 10 patients studied herein, it may be concluded that HBsAg335-343 is an HBV group specific CTL epitope. The same was not true for HBsAg348-357, however, since only seven of the ten patients were found to be infected by viruses that encode the prototype sequence used for in vitro stimulation (GLSPTVWLSV (SEQ ID NO:26)). The

remaining three patients (A-9, A-10, A-13) displayed a variant sequence in which the carboxy-terminal valine was substituted by alanine at position 357. Among the patients infected by the prototype virus, CTL responders and nonresponders to HBsAg₃₄₈₋₃₅₇ were observed, just as for the response to BsAg₃₃₅₋₃₄₃. On the other hand, none of the 3 patients infected by the variant virus displayed a CTL response to the prototype peptide.--

Please replace the paragraph beginning at page 40, line 26, with the following:

--An HBsAg₃₃₅₋₃₄₃ specific CTL line (patient A-1) and an HBsAg₃₄₈₋₃₅₇ specific CTL line (patient A-4) were generated by stimulation with peptide sequences WLSLLVPFV (SEQ ID NO:10) and GLSPTVWLSV (SEQ ID NO:26), respectively. CTL were incubated with ⁵¹Cr-labelled JY target cells that had been preincubated either with media, with the inducing peptide or (in the case of HBsAg₃₄₈₋₃₅₇) with a variant peptide (GLSPTVWLSA (SEQ ID NO:57)). CTL were also incubated with ⁵¹Cr-labelled JY target cells that had been infected with a panel of 6 recombinant vaccinia viruses that express the HBV major (V-HBs), middle (V-preS2), and large (V-preS1) envelope polypeptides derived from ayw and adw HBV genomes. Wild-type vaccinia viruses (V-wt) were used as controls. The HBsAg₃₃₅₋₃₄₃ specific CTL line (right panel) was used at an E:T=40:1. The HBsAg₃₄₈₋₃₅₇ specific CTL line (left panel) was used at an E:T=3:1. Results shown represent % lysis in a 4 hour ⁵¹Cr release assay.--

Please cancel the present "SEQUENCE LISTING", pages 42-49, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 18, at the end of the application. Cancel the page numbers for the Claims and Abstract and renumber as pages 42-44, accordingly.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 22 of page 5 has been amended as follows:

Fig. 1 shows that HBsAg335-343, WLSLLVPFV (SEQ ID NO:10), is the minimal optimal CTL epitope recognized by CTL stimulated HBsAg329-348. A CTL clone from patient A-1 and a CTL cloned line from patient A-3, generated by stimulation with HBsAg329-348, were tested against JY target cells prepulsed either with truncations (SEQ ID NOS:22 and 28-39, respectively) (upper panels) or with overlapping 9-mers or 10-mers (SEQ ID NOS:22, 40-50, 15, 10, 16, 51-55, 25, 56, 38 and 39, respectively) (lower panels) covering HBsAg329-348.

Paragraph beginning at line 29 of page 5 has been amended as follows:

Fig. 2 further confirms that an optimal epitope within HBsAg329-348 for in vitro CTL induction is HBsAg335-343 (SEQ ID NOS:22, 53, 52, 10, 51, 50 and 25, respectively).

Paragraph beginning at line 3 of page 6 has been amended as follows:

Fig. 4 shows the results of HLA-A2.1 competitive binding inhibition assays (SEQ ID NOS:24, 7, 9, 10, 25, 26 and 27, respectively), represented as % inhibition of HBcAg18-27 specific lysis in a 4 hour ⁵¹Cr release assay.

Paragraph beginning at line 23 of page 33 has been amended as follows:

Two HLA-A2 positive patients with acute hepatitis (A-1 and A-3) were initially selected for analysis of the CTL response to HbsAg329-348 (ASARFSWLSLLVPFVQWFGV (SEQ ID NO:22) (~~Seq ID No. 22~~)), which contains 2 overlapping HLA a2.1 allele specific binding motifs (WLSLLVPFV (SEQ ID NO:10) and LLVPFVQWFGV (SEQ ID NO:25)). One of these patients (A-3) was known from previous experiments to display an HLA A2 restricted CTL response to a 10 residue HBV nucleocapsid epitope (HBcAg18-27) that also represents an HLA A2.1 allele specific binding motif (FLPSDFFPSV (SEQ ID NO:23)). This patient was considered a potential responder to one or both of the motifs in HBsAg329-348. Another patient (A-1), known to be a nonresponder to HBsAg18-27, was studied for comparison.

Paragraph beginning at line 6 of page 35 has been amended as follows:

The results, shown in Fig. 1, indicated that only the first of the HLA-A2.1 binding motifs (HBsAg335-343) is recognized by the CTL. Furthermore, the data demonstrate that this peptide (WLSLLVPFV (SEQ ID NO:10)) is the minimal, optimal HLA-A2 restricted epitope recognized by HBsAg329-348 stimulated CTL, since omission of the extreme amino-terminal or the extreme carboxy-terminal residue from HBsAg335-343 abolishes recognition by the CTL.

Paragraph beginning at line 22 of page 37 has been amended as follows:

Nucleotide sequence analysis of circulating virion DNA in acutely infected patients showed that all patients, including the CTL nonresponders, were infected by viruses that expressed the precise amino acid sequence present in the prototype HBsAg335-343 peptide used to stimulate expansion of CTL in vitro. Since residues 335-343 are known to be conserved in all the published HBV sequences derived

from all 4 HBV subtypes, as published in the GenBank-72 database, as well as in the 10 patients studied herein, it may be concluded that HBsAg₃₃₅₋₃₄₃ is an HBV group specific CTL epitope. The same was not true for HBsAg₃₄₈₋₃₅₇, however, since only seven of the ten patients were found to be infected by viruses that encode the prototype sequence used for in vitro stimulation (GLSPTVWLSV (SEQ ID NO:26)). The remaining three patients (A-9, A-10, A-13) displayed a variant sequence in which the carboxy-terminal valine was substituted by alanine at position 357. Among the patients infected by the prototype virus, CTL responders and nonresponders to HBsAg₃₄₈₋₃₅₇ were observed, just as for the response to BsAg₃₃₅₋₃₄₃. On the other hand, none of the 3 patients infected by the variant virus displayed a CTL response to the prototype peptide.

Paragraph beginning at line 26 of page 40 has been amended as follows:

An HBsAg₃₃₅₋₃₄₃ specific CTL line (patient A-1) and an HBsAg₃₄₈₋₃₅₇ specific CTL line (patient A-4) were generated by stimulation with peptide sequences WLSLLVPFV (SEQ ID NO:10) and GLSPTVWLSV (SEQ ID NO:26), respectively. CTL were incubated with ⁵¹Cr-labelled JY target cells that had been preincubated either with media, with the inducing peptide or (in the case of HBsAg₃₄₈₋₃₅₇) with a variant peptide (GLSPTVWLSA (SEQ ID NO:57)). CTL were also incubated with ⁵¹Cr-labelled JY target cells that had been infected with a panel of 6 recombinant vaccinia viruses that express the HBV major (V-HBs), middle (V-preS2), and large (V-preS1) envelope polypeptides derived from ayw and adw HBV genomes. Wild-type vaccinia viruses (V-wt) were used as controls. The HBsAg₃₃₅₋₃₄₃ specific CTL line (right panel) was used at an E:T=40:1. The HBsAg₃₄₈₋₃₅₇ specific CTL line (left panel) was used at an E:T=3:1. Results shown represent % lysis in a 4 hour ⁵¹Cr release assay.



RECEIVED

DEC 30 2003

TECH CENTER 1600/2900

TO THE U.S. PATENT AND TRADEMARK OFFICE

Please stamp the date of receipt of the following document(s) and return this card to us:

INVENTOR(S):	Francis V. Chisari
RE:	PATENT APPLICATION FILED 5/21/01 FOR "PEPTIDES FOR INDUCING CYTOTOXIC T LYMPHOCYTE RESPONSES TO HEPATITIS B VIRUS"
TITLE OF DOCUMENT(S):	Communication Under 37 CFR 1.821-1.825 and Amendment; Sequence Listing paper copy pages 1-18; Sequence Listing diskette copy; copy of Notice to Comply Transmittal Form PTO/SB 21;
Application No.	09/863,054
File No.	14740-0004-21
Date Due	2 Nov. 2002
Date Mailed	30 Oct. 2002
Attorney/Secretary	JML/mcd

